

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|   |           |  |
|---|-----------|--|
| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>C12Q 1/68, G01N 33/50</b>  | <b>A1</b> | <b>(11) International Publication Number:</b> <b>WO 99/23254</b><br><b>(43) International Publication Date:</b> 14 May 1999 (14.05.99)   |
| <b>(21) International Application Number:</b> PCT/US98/22966<br><b>(22) International Filing Date:</b> 30 October 1998 (30.10.98)<br><b>(30) Priority Data:</b><br>60/063,857 31 October 1997 (31.10.97) US<br><b>(71) Applicant (for all designated States except US):</b><br>AFFYMETRIX, INC. [US/US]; 3380 Central Expressway,<br>Santa Clara, CA 95051 (US).<br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only):</b> LOCKHART, David, J.<br>[US/US]; 610 Mountain View Avenue, Mountain View, CA<br>94041 (US). NAIR, Archana [IN/US]; 3589 Lochinvar Av-<br>enue, No. 6, Santa Clara, CA 95051 (US). WARRINGTON,<br>Janet, A. [US/US]; 325 Thompson Avenue, Mountain View,<br>CA 94043 (US).<br><b>(74) Agents:</b> KAGAN, Sarah, A. et al.; Banner & Witcoff, Ltd., 11th<br>floor, 1001 G Street, N.W., Washington, DC 20001-4597<br>(US). |           | <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR,<br>BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE,<br>GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ,<br>LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW,<br>MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,<br>TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO<br>patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian<br>patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European<br>patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR,<br>IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF,<br>CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>With international search report.</i><br><i>Before the expiration of the time limit for amending the<br/>claims and to be republished in the event of the receipt of<br/>amendments.</i> |
| <b>(54) Title:</b> EXPRESSION PROFILES IN ADULT AND FETAL ORGANS<br><br><b>(57) Abstract</b><br><br>Expression profiles have been constructed for liver and brain, in adult and fetal and adolescent tissue. These provide the art with probes which are highly differentially expressed among stages and organs. The profiles and probes can be used to prioritize potential drug targets, to monitor disease progression and remission, and to assess drug metabolism. Solid supports are also provided which facilitate these uses.  |           |  |

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |  |    |  |    |                          |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania                  | ES | Spain                                    | LS | Lesotho                                      | SI | Slovenia                 |
| AM | Armenia                  | FI | Finland                                  | LT | Lithuania                                    | SK | Slovakia                 |
| AT | Austria                  | FR | France                                   | LU | Luxembourg                                   | SN | Senegal                  |
| AU | Australia                | GA | Gabon                                    | LV | Latvia                                       | SZ | Swaziland                |
| AZ | Azerbaijan               | GB | United Kingdom                           | MC | Monaco                                       | TD | Chad                     |
| BA | Bosnia and Herzegovina   | GE | Georgia                                  | MD | Republic of Moldova                          | TG | Togo                     |
| BB | Barbados                 | GH | Ghana                                    | MG | Madagascar                                   | TJ | Tajikistan               |
| BE | Belgium                  | GN | Guinea                                   | MK | The former Yugoslav<br>Republic of Macedonia | TM | Turkmenistan             |
| BF | Burkina Faso             | GR | Greece                                   |    |  | TR | Turkey                   |
| BG | Bulgaria                 | HU | Hungary                                  | ML | Mali   | TT | Trinidad and Tobago      |
| BJ | Benin                    | IE | Ireland                                  | MN | Mongolia                                     | UA | Ukraine                  |
| BR | Brazil                   | IL | Israel                                   | MR | Mauritania                                   | UG | Uganda                   |
| BY | Belarus                  | IS | Iceland                                  | MW | Malawi                                       | US | United States of America |
| CA | Canada                   | IT | Italy                                    | MX | Mexico                                       | UZ | Uzbekistan               |
| CF | Central African Republic | JP | Japan                                    | NE | Niger  | VN | Viet Nam                 |
| CG | Congo                    | KE | Kenya                                    | NL | Netherlands                                  | YU | Yugoslavia               |
| CH | Switzerland              | KG | Kyrgyzstan                               | NO | Norway                                       | ZW | Zimbabwe                 |
| CI | Côte d'Ivoire            | KP | Democratic People's<br>Republic of Korea | NZ | New Zealand                                  |    |                          |
| CM | Cameroon                 |    |  | PL | Poland                                       |    |                          |
| CN | China                    | KR | Republic of Korea                        | PT | Portugal                                     |    |                          |
| CU | Cuba                     | KZ | Kazakhstan                               | RO | Romania                                      |    |                          |
| CZ | Czech Republic           | LC | Saint Lucia                              | RU | Russian Federation                           |    |                          |
| DE | Germany                  | LI | Liechtenstein                            | SD | Sudan  |    |                          |
| DK | Denmark                  | LK | Sri Lanka                                | SE | Sweden                                       |    |                          |
| EE | Estonia                  | LR | Liberia                                  | SG | Singapore                                    |    |                          |

## EXPRESSION PROFILES IN ADULT AND FETAL ORGANS

### BACKGROUND OF THE INVENTION

Expression profiles of genes in particular organs or disease states or developmental stages provide molecular tools for evaluating toxicity, drug efficacy,  
5 drug metabolism, development, and disease monitoring. Changes in the expression profile from a baseline profile can be used as an indication of such effects.

### SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method of screening a drug for deleterious side effects on a cell.

10 It is another object of the present invention to provide a method of distinguishing between a fetal and an adult liver sample.

It is an object of the present invention to provide a method of distinguishing between a fetal and adult brain tissue.

15 Another object of the invention is to provide a method of determining the source of a tissue as adult brain or adult liver.

Another object of the invention is to provide a method of distinguishing a tissue source as fetal brain or fetal liver.

Another object of the invention is to provide a solid support for screening a drug for deleterious side effects on a cell.

20 Another object of the invention is to provide a solid support for distinguishing between a fetal and an adult liver sample.

Still another object of the invention is to provide a solid support for distinguishing between a fetal and adult brain tissue.

- 2 -

Yet another object of the invention is to provide a solid support for determining the source of a tissue as adult brain or adult liver.

Another object of the invention is to provide a solid support for distinguishing a tissue source as fetal brain or fetal liver.

5           These and other objects of the invention are achieved by providing a method of screening a drug for deleterious side effects on a cell. The method comprises the step of:

          assaying for the amount of expression in the cell of two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagmin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, 10           apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, cytochrome p450-2E1, and thymosin beta-10, wherein the expression in the cell is assayed before and after the cell has been contacted with the drug, wherein alteration of the amount of expression of at least one of these genes by 15           the drug is indicative of a deleterious side effect.

          According to another embodiment of the invention a method of distinguishing between a fetal and an adult liver sample is provided. The method comprises the step of:

20           assaying for expression in the sample of two or more genes selected from the group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and thymosin beta-10, wherein expression of G6PD, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, cytochrome p450-2E1, and thymosin 25           beta-10 are indicative of an adult liver, and expression of calmodulin, hepatocyte gf, IGF binding protein 1, and ubiquitin are indicative of a fetal liver.

          According to still another embodiment of the invention a method of distinguishing between a fetal and adult brain tissue is provided. The method comprises the step of:

30           assaying for expression in the tissue of two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and thymosin beta-10,

- 3 -

wherein expression of nicotinic acetylcholine receptor beta 2 or ubiquitin indicates an adult brain.

Another aspect of the invention is a method of determining the source of a tissue as adult brain or adult liver. The method comprises the steps of:

5        assaying for expression in the tissue of two or more genes selected from the group consisting of: calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and cytochrome  
10        p450-2E1, wherein expression of calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, ubiquitin, or bone morphogenetic protein precursor indicates a brain source for the tissue and wherein expression of pyruvate dehydrogenase E1, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, apolipoprotein B100, thymosin beta-10, hepatocyte gf, IGF binding  
15        protein 1, or cytochrome p450-E1 indicates a liver source for the tissue.

Still another aspect of the invention is a method of distinguishing a tissue source as fetal brain or fetal liver. The method comprises the step of:

      assaying for expression in the tissue of two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagamin, neuromodulin, pyruvate  
20        dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and thymosin beta-10, wherein expression of G6PD, calcium channel, synaptotagamin, neuromodulin, thymosin beta-10 or bone morphogenetic protein precursor indicates a fetal brain source and expression of pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF  
25        binding protein 1, ubiquitin, indicates a fetal liver source.

Another embodiment of the invention provides a solid support for screening a drug for deleterious side effects on a cell. The solid support comprises: at least two oligonucleotides for probing two or more genes selected from the group consisting of:  
30        G6PD, calcium channel, synaptotagamin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF

- 4 -

binding protein 1, ubiquitin, bone morphogenetic protein precursor, cytochrome p450-2E1, and thymosin beta-10, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

Yet another aspect of the invention is a solid support for distinguishing between a fetal and an adult liver sample. The solid support comprises: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and thymosin beta-10 wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

In another aspect of the invention a solid support for distinguishing between a fetal and adult brain tissue is provided. The solid support comprises: two more oligonucleotides for detecting two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and thymosin beta-10, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

In still another aspect of the invention a solid support for determining the source of a tissue as adult brain or adult liver is provided. The solid support comprises:

two or more oligonucleotides for detecting two or more genes selected from the group consisting of: calcium channel, synaptotagmin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and cytochrome p450-2E1, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

Another embodiment of the invention is a solid support for distinguishing a tissue source as fetal brain or fetal liver. The solid support comprises: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagmin, neuromodulin, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone

- 5 -

morphogenetic protein precursor, and thymosin beta-10, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows a plot of the fetal brain hybridization intensities, poly(A)<sup>+</sup> versus cDNA yielded a line with a slope of 1.

#### **DETAILED DESCRIPTION**

Genes have been found whose expression is varied greatly ( $\geq 10$  fold) between different developmental stages and between different organs in the same developmental stages. Thus these genes can be used as interrogators (probes) to determine the expression pattern of unknown cells or samples to identify them as belonging to the appropriate developmental stage or organ source. The genes are listed in Table 4. The complete sequences of the genes are available from GenBank using the accession numbers shown in the table.

Any selection of at least 2 through 16 of the genes can be used as interrogators. For a particular interrogation of two conditions or sources, it is desirable to select those genes which display a great deal of difference in the expression pattern between the two conditions or sources. At least a two-fold difference is desirable, but a five-fold or ten-fold difference is preferred.

Interrogations of the genes or proteins can be performed to yield different information. Potential drugs can be screened to determine if the expression of these genes is inappropriately altered. This may be useful, for example, in determining whether a particular drug is prescribed to a pregnant woman. In the case where a fetally expressed gene's expression is affected by the potential drug, prohibition of the drug to pregnant woman is indicated. Similarly, a drug which causes expression of a gene which is not normally expressed by a fetus, should be prohibited to pregnant woman.

Molecular expression markers for either brain or liver can be used to confirm tissue source identifications made on the basis of morphological criteria. In some situations, identifications of cell type or source is ambiguous based on classical criteria. In these situations the molecular expression markers of the present invention are useful.

In addition, disease progression involving either the brain or the liver can be monitored by following the expression patterns of the involved organs using the

- 6 -

molecular expression markers of the present invention. Perturbed expression can be observed in the diseased state. Monitoring of the efficacy of certain drug regimens can also be accomplished by following the expression patterns of the molecular expression markers.

5           Although only a few different developmental time points have been observed, as shown in the examples below, other developmental stages can be studied using these same molecular expression markers. The importance of these markers in development has been shown here, however, variations in their expression may occur at other times. For example, one could study the expression of these markers at other gestational  
10 stages, at birth, postnatally, and throughout the human life cycle.

          Solid supports containing oligonucleotide probes for differentially expressed genes can be filters, polyvinyl chloride dishes, etc. Any solid surface to which oligonucleotides can be bound, either directly or indirectly, either covalently or non-covalently, can be used. A preferred solid support is a high density array or DNA chip.  
15 These contain a particular oligonucleotide probe in a predetermined location on the array. Each predetermined location may contain more than one molecule of the probe, but each molecule within the predetermined location has an identical sequence. Such predetermined locations are termed features. There may be, for example, from 2, 10, 100, 1000, to 10,000, 100,000, or 400,000 of such features on a single solid support.  
20 The solid support, or the area within which the probes are attached may be on the order of a square centimeter.

          Oligonucleotide probe arrays for expression monitoring can be made and used according to any techniques known in the art. See for example, Lockhart, D.J. *et al.*, *Nature Biotechnology* 14:1675-1680 (1996) and McGall, G. *et al.*, *Proc. Nat.Acad.Sci. USA* 93:13555-13460 (1996).  
25

          The genes which are assayed or interrogated according to the present invention are typically in the form of mRNA or reverse transcribed mRNA. The genes may be cloned or not. The genes may be amplified or not. The cloning itself does not appear to bias the representation of genes within a population. However, it may be preferable  
30 to use polyA+ RNA as a source, as it can be used with less processing steps. The sequences of the expression marker genes are in the public databases. Table 4 provides



- 7 -

the accession numbers for the sequences. The sequences of the genes in GenBank are expressly incorporated herein. Some of the genes are also the subject of scientific and learned journal articles. These include: Anand,R. and Lindstrom,J., Nucleotide sequence of the human nicotinic acetylcholine receptor beta 2 subunit gene, Nucleic Acids Res. 18 (14), 4272 (1990) (MEDLINE 90332444); Takizawa,T., Huang,I.Y., Ikuta,T. and Yoshida,A., Human glucose-6-phosphate dehydrogenase: primary structure and cDNA cloning, Proc. Natl. Acad. Sci. U.S.A. 83 (12), 4157-4161 (1986) (MEDLINE 86233391); Karathanasis,S.K., Apolipoprotein multigene family: tandem organization of human apolipoprotein AI, CIII, and AIV genes, Proc. Natl. Acad. Sci. U.S.A. 82 (19), 6374-6378 (1985) (MEDLINE 86016704); Sakai,J., Hoshino,A., Takahashi,S., Miura,Y., Ishii,H., Suzuki,H., Kawarabayasi,Y. and Yamamoto,T., Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene, J. Biol. Chem. 269 (3), 2173-2182 (1994) (MEDLINE 94124575); Schuppan,D., Cantaluppi,M., Becker,J., Veit,A., Bunte,T., Troyer,D., Schuppan,F., Schmid,M., Ackermann,R. and Hahn,E., Undulin, an Extracellular Matrix Glycoprotein Associated with Collagen Fibrils, J. Biol. Chem. 265, 8823-8832 (1990) (MEDLINE 90256812); Just,M., Herbst,H., Hummel,M., Durkop,H., Tripier,D., Stein,H. and Schuppan,D., Undulin is a novel member of the fibronectin-tenascin family of extracellular matrix glycoproteins, J. Biol. Chem. 266, 17326-17332 (1991)(MEDLINE 91373351); Ehrenborg,E., Larsson,C., Stern,I., Janson,M., Powell,D.R. and Luthman,H., Contiguous localization of the genes encoding human insulin-like growth factor binding proteins 1 (IGBP1) and 3 (IGBP3) on chromosome 7, Genomics 12 (3), 497-502 (1992) (MEDLINE 92217971); Kosik,K.S., Orecchio,L.D., Bruns,G.A., Benowitz,L.I., MacDonald,G.P., Cox,D.R. and Neve,R.L., Human GAP-43: its deduced amino acid sequence and chromosomal localization in mouse and human, Neuron 1 (2), 127-132 (1988)(MEDLINE 90166498; Perin,M.S., Johnston,P.A., Ozcelik,T., Jahn,R., Francke,U. and Sudhof,T.C., Structural and functional conservation of synaptotagmin (p65) in Drosophila and humans, J. Biol. Chem. 266 (1), 615-622 (1991) (MEDLINE 91093190); Fischer,R., Koller,M., Flura,M., Mathews,S., Strehler-Page,M.A., Krebs,J., Penniston,J.T., Carafoli,E. and Strehler,E.E., Multiple divergent mRNAs code for a single human calmodulin, J. Biol.

- 8 -

Chem. 263 (32), 17055-17062 (1988)(MEDLINE 89034207). Each of these articles is also expressly incorporated herein.

Glucose-6-phosphate dehydrogenase deficiency can lead to significant hemolysis. Hemolytic crisis can be induced by exposure to an oxidant, producing profound drops in hematocrit and hemoglobin levels. Calmodulin is a calcium binding protein which controls the assembly of myosin molecules. Calcium channel is involved in ischemic heart disease, stroke and neuronal development and transmission. Neuromodulin is also known as GAP-43. It is involved in neuronal development. Prooncprotein EWS binds to neuromodulin. VLDLR is the very low density lipoprotein receptor. It is involved in hyper-cholesterolemia. Undulin is involved with kidney diseases, kidney transplantation and hemodialysis. (Clin Nephrol 44(3), 178-184 (1995)). It is also involved in schistosomiasis and alcoholic liver cirrhosis. Hepatogastroenterology 42(1), 22-26 (1995). Pyruvate dehydrogenase E1 deficiency is associated with Leigh syndrome, microcephaly, and motor neuropathy. Apolipoprotein B100 is involved in atherosclerosis and hypercholesterolemia. Hepatocyte growth factor (gf) is involved in stimulation of the growth of hepatocytes. Insulin-like growth factor-binding protein (IGFBP-3) predisposes breast cancer cells to programmed cell death. Ubiquitin is involved in dendrite outgrowth and differentiation. Cytochrome P450 2E1 is the principal catalyst of human oxidative halothane metabolism in vitro. (J Pharmacol Exp Ther 281(1), 400-411 (1997)). Thymosin beta-10 is detected mainly in malignant breast tissue, particularly in the cancerous cells, whereas the normal cell population around the lesions shows very weak staining. Also, the intensity of staining in the cancerous cells was proportionally increased with the increasing grade of the lesions. (Br J Cancer 1996 Nov;74(9):1441-1444). In addition, in the highly metastatic human melanoma cell line, BLM, thymosin beta-10 correlated with the malignant phenotype. (Biochem Biophys Res Commun 1996 Aug 23;225(3):808-816.)

The protein product of the gene can be assayed to determine the amount of expression. Methods for assaying for a protein include Western blot, immunoprecipitation, radioimmunoassay. However, it is preferred according to the invention that the mRNA be assayed as an indication of expression. Methods for

- 9 -

assaying for mRNA include Northern blots, slot blots, dot blots, and hybridization to an ordered array of oligonucleotides. Any method for specifically and quantitatively measuring a specific protein or mRNA or DNA product can be used.

5 Oligonucleotide probes for interrogating the tissue or cell sample are preferably of sufficient length to specifically hybridize only to appropriate, complementary genes or transcripts. Typically the oligonucleotide probes will be at least 10, 12, 14, 16, 18, 20 or 25 nucleotides in length. In some cases longer probes of at least 30, 40, or 50 nucleotides will be desirable.

10 The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

#### EXAMPLE 1

15 Gene expression levels were quantitatively measured in human poly(A)+ and cDNA libraries using high density oligonucleotide arrays containing probes for more than 6500 human genes. Probe arrays (DNA chips) of this type have been shown to behave quantitatively with high specificity and sensitivity (Lockhart, D.J. et al., 1996, Nat. Biotech. 14:1657-1680). The arrays were designed and synthesized based on sequence information obtained from GenBank and dbEST. Half the genes were  
20 selected from full-length human sequences in Gen Bank and the remaining half were selected from clustered ESTs that have sequence similarity to genes of known function. Samples derived from adult brain, adult liver, fetal brain and fetal liver were hybridized to the probe arrays and the relative concentration of more than 6500 human genes were measured simultaneously. The RNAs were classified by relative abundance and  
25 differentially expressed genes were identified by direct comparison. The following comparisons were made; 1) fetal brain:adult brain, 2) fetal liver:adult liver, 3) adult brain:adult liver, 4) fetal brain:fetal liver. Using this method one can produce, in a relatively short period of time, a quantitative representation of gene expression for the major organs of the human body.

- 10 -

EXAMPLE 2

The sources of the samples interrogated are shown in Table 1. The samples were obtained commercially.

- 11 -

TABLE 1

Starting Material

| TISSUE             | SOURCE | DESCRIPTION      | CAT. #     |
|--------------------|--------|------------------|------------|
| <b>Human Brain</b> |        |                  |            |
| Fetal              | cDNA   | F&M, 19-23 wks   | Str 937226 |
| Fetal              | PolyA  | F&M, 20-25 wks   | Cl 6525-1  |
| Adult              | PolyA  | F & M, 37-60 yrs | Cl 6516-1  |
| <b>Human Liver</b> |        |                  |            |
| Adult              | cDNA   | M, 49 yrs        | Str 937224 |
| Adolescent Liver   | PolyA  | F, 15 yrs        | Cl 6510-1  |
| Fetal              | PolyA  | F & M, 16-32 wks | Cl 6527-1  |

- 12 -

### EXAMPLE 3

The profiles using cloned brain cDNA and brain poly(A)<sup>+</sup> mRNA (reverse transcribed) as samples were compared. As shown in Table 2, each sample type yielded a large number of low copy messages. The percentage of the unique messages which were low copy messages was about the same for each sample source.

5

As shown in Figure 1, a plot of the fetal brain hybridization intensities, poly(A)<sup>+</sup> versus cDNA yielded a line with a slope of 1.

**TABLE 2****cDNA and Poly(A) Brain Message Comparison**

|                      | <b>cDNA - Poly(A)</b> |
|----------------------|-----------------------|
| <b>Common</b>        | <b>1854</b>           |
| <b>Unique(cDNA)</b>  | <b>680</b>            |
| <b>Low copy</b>      | <b>500(0.74)</b>      |
| <b>Unique(polyA)</b> | <b>329</b>            |
| <b>Low Copy</b>      | <b>239(0.73)</b>      |

- 14 -

EXAMPLE 4

5 The mRNA in brain and liver were compared (via their cDNA). Table 3 shows the results. The first column of data compares fetal brain and fetal liver. The second column of data compares fetal brain and adult brain. The third column of data compares the adolescent liver with the fetal liver. The percentage of low copy messages in fetal liver is very low. This indicates that the public databases (from which the probes were derived) contain an under representation of messages from fetal liver.



**TABLE 3**  
**Common and Unique mRNA in Brain and Liver**

|              | FBA - FLB (%) | FBA - ABB (%) | AdLA - FLB (%) |
|--------------|---------------|---------------|----------------|
| Common (A+B) | 1176          | 1244          | 1268           |
| Unique (A)   | 1035          | 941           | 547            |
| Low copy (A) | 691 (0.67)    | 706 (0.75)    | 279 (0.51)     |
| Unique (B)   | 651           | 159           | 534            |
| Low copy (B) | 51 (0.08)     | 82 (0.52)     | 157 (0.29)     |

- 16 -

EXAMPLE 5

The results of the comparison of expression levels for 16 particular genes in fetal and adult, lung and brain, are shown in Table 4.

TABLE 4

| Accession number | Gene name                               | Intensity Abbrain | Intensity Fbrain | Intensity Aliver | Intensity Fliver |         |
|------------------|---|-------------------|------------------|------------------|------------------|---------|
| M12996           | G6PD                                    | 210               | 180              | 70               | 0                | G6PD    |
| T53360           | calcium channel                         | 490               | 350              | 0                | 0                | CACH    |
| M55047           | synaptotagamin                          | 1380              | 430              | 0                | 0                | SYNTGN  |
| M25667           | neuromodulin                            | 1500              | 2860             | 0                | 0                | NEUM    |
| J04046           | calmodulin                              | 1500              | 1510             | 0                | 1970             | CAM     |
| X53179           | nicotinic acetylcholine receptor beta 2 | 1430              | 0                | 0                | 0                | NACB2   |
| D16532           | VLDLR                                   | 0                 | 0                | 1300             | 0                | VLDLR   |
| M64108           | udulin 1/undulin/excellmatr glycopro    | 0                 | 0                | 490              | 0                | XCMG/UN |
|                  |   |                   |                  |                  |                  |         |
| T65758           | pyruvate dehydrogenase E1               | 230               | 230              | 5400             | 2290             | PYDHE1  |
| M10373           | apolipoprotein B100                     | 0                 | 0                | 8520             | 22850            | APOB100 |
| R85613           | hepalocyte gf                           | 0                 | 0                | 400              | 3790             | HPGF    |
| M74587           | IGF binding pro1                        | 0                 | 0                | 570              | 29520            | IGFBP1  |
| T88723           | ubiquitin                               | 6900              | 0                | 300              | 42490            | UBQ     |
| R77212           | bone morphogenetic pro precursor        | 10800             | 5560             | 0                | 0                | BMPP    |
| H46990           | cytochrome p450 - 2E1                   | 0                 | 0                | 10510            | 0                | CYP2E1  |
| T63133           | thymosin beta-10                        | 550               | 3840             | 2420             | 0                | THYB10  |

CLAIMS

1. A method of screening a drug for deleterious side effects on a cell comprising the steps of:

5        assaying for the amount of expression in the cell of two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagmin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, cytochrome p450-2E1, and thymosin beta-10, wherein  
10       the expression in the cell is assayed before and after the cell has been contacted with the drug, wherein alteration of the amount of expression of at least one of these genes by the drug is indicative of a deleterious side effect.

2. A method of distinguishing between a fetal and an adult liver sample comprising the steps of:

15       assaying for expression in the sample of two or more genes selected from the group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and thymosin beta-10, wherein expression of G6PD, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, cytochrome p450-2E1, and thymosin  
20       beta-10 are indicative of an adult liver, and expression of calmodulin, hepatocyte gf, IGF binding protein 1, and ubiquitin are indicative of a fetal liver.

3. A method of distinguishing between a fetal and adult brain tissue, comprising the steps of:

25       assaying for expression in the tissue of two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and thymosin beta-10, wherein expression of nicotinic acetylcholine receptor beta 2 or ubiquitin indicates an adult brain.

4. A method of determining the source of a tissue as adult brain or adult liver, comprising the steps of:

- 19 -

assaying for expression in the tissue of two or more genes selected from the group consisting of: calcium channel, synaptotagmin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and cytochrome p450-2E1, wherein expression of calcium channel, synaptotagmin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, ubiquitin, or bone morphogenetic protein precursor indicates a brain source for the tissue, and wherein expression of pyruvate dehydrogenase E1, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, apolipoprotein B100, thymosin beta-10, hepatocyte gf, IGF binding protein 1, or cytochrome p450-E1 indicates a liver source for the tissue.

5. A method of distinguishing a tissue source as fetal brain or fetal liver, comprising the steps of:

assaying for expression in the tissue of two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagmin, neuromodulin, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and thymosin beta-10, wherein expression of G6PD, calcium channel, synaptotagmin, neuromodulin, thymosin beta-10 or bone morphogenetic protein precursor indicates a fetal brain source and expression of pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, indicates a fetal liver source.

6. The method of claim 1, 2, 3, 4, or 5, wherein expression of at least 3 genes are assayed.

7. The method of claim 1, 2, 4, or 5, wherein expression of at least 4 genes are assayed.

8. The method of claim 1, 2, 4, or 5, wherein expression of at least 5 genes are assayed.

9. The method of claim 1, 2, 4, or 5, wherein expression of at least 6 genes are assayed.

10. The method of claim 1, 2, 4, or 5, wherein expression of at least 7 genes are assayed.

- 20 -

11. The method of claim 1, 2, 4, or 5, wherein expression of at least 8 genes are assayed.

12. The method of claim 1, 2, 4, or 5, wherein expression of at least 9 genes are assayed.

5 13. The method of claim 1, 4, or 5, wherein expression of at least 10 genes are assayed.

14. A solid support for screening a drug for deleterious side effects on a cell comprising: at least two oligonucleotides for probing two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagmin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, cytochrome p450-2E1, and thymosin beta-10, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

15 15. A solid support for distinguishing between a fetal and an adult liver sample comprising: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calmodulin, VLDLR, udulin1/undulin/extracellular matrix glycoprotein, hepatocyte gf, IGF binding protein 1, ubiquitin, cytochrome p450-2E1, and thymosin beta-10 wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

20 16. A solid support for distinguishing between a fetal and adult brain tissue, comprising: two more oligonucleotides for detecting two or more genes selected from the group consisting of: nicotinic acetylcholine receptor beta 2, ubiquitin, and thymosin beta-10, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

25 17. A solid support for determining the source of a tissue as adult brain or adult liver, comprising:

30 two or more oligonucleotides for detecting two or more genes selected from the group consisting of: calcium channel, synaptotagmin, neuromodulin, calmodulin, nicotinic acetylcholine receptor beta 2, VLDLR, udulin1/undulin/extracellular matrix

- 21 -

glycoprotein, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and cytochrome p450-2E1, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

5           18.    A solid support for distinguishing a tissue source as fetal brain or fetal liver, comprising: two or more oligonucleotides for detecting two or more genes selected from the group consisting of: G6PD, calcium channel, synaptotagmin, neuromodulin, pyruvate dehydrogenase E1, apolipoprotein B100, hepatocyte gf, IGF binding protein 1, ubiquitin, bone morphogenetic protein precursor, and thymosin beta-  
10           10, wherein each oligonucleotide comprises a sequence which is complementary to one of the two or more genes.

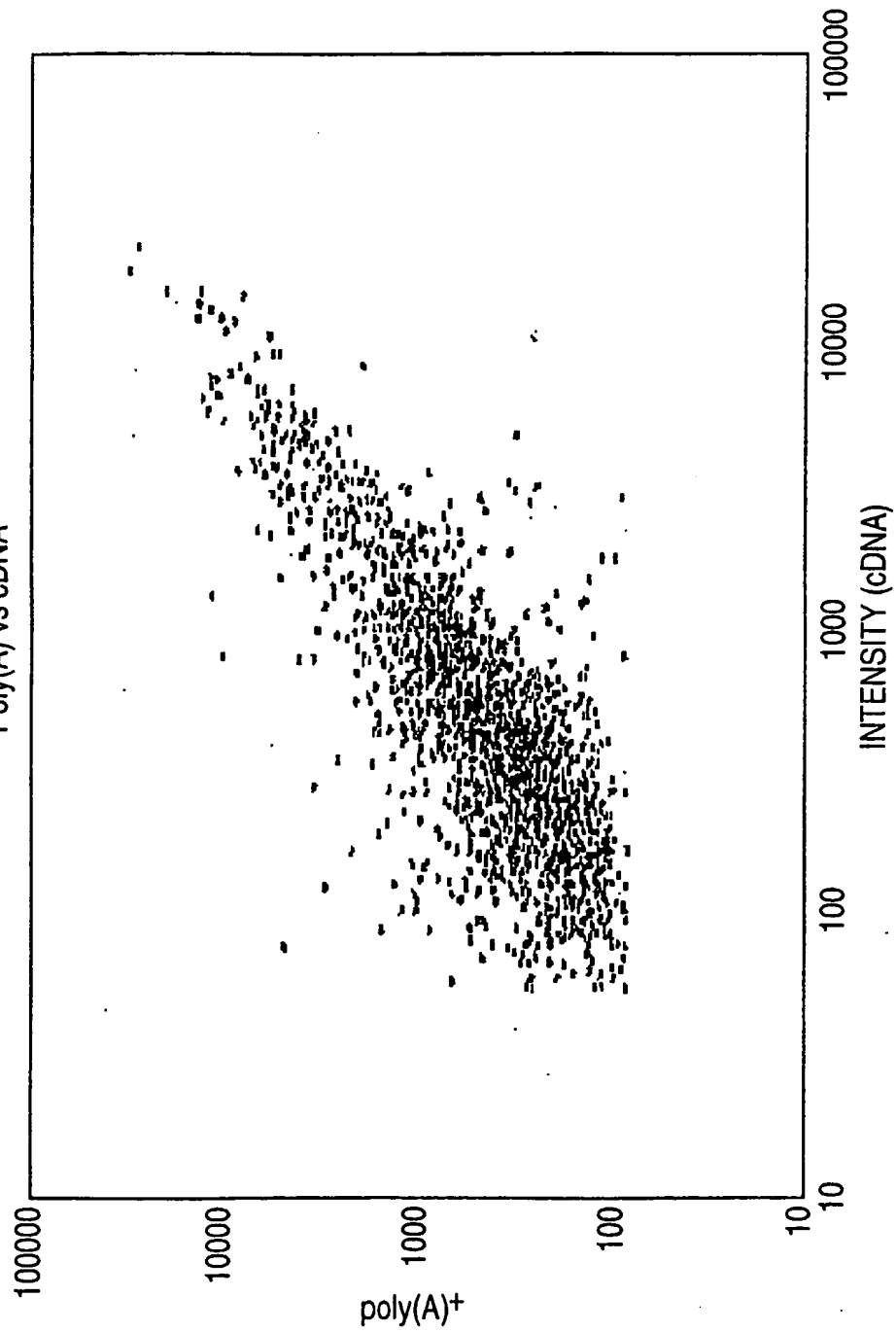
          19.    The solid support of claim 14, 15, 16, 17, or 18 which is an array comprising at least 10 different oligonucleotides in discrete locations per cm<sup>2</sup>.

          20.    The solid support of claim 14, 15, 16, 17, or 18 which is an array  
15           comprising at least 100 different oligonucleotides in discrete locations per cm<sup>2</sup>.

          21.    The solid support of claim 14, 15, 16, 17, or 18 which is an array comprising at least 1,000 different oligonucleotides in discrete locations per cm<sup>2</sup>.

          22.    The solid support of claim 14, 15, 16, 17, or 18 which is an array  
20           comprising at least 10,000 different oligonucleotides in discrete locations per cm<sup>2</sup>.

1/1

**FIG. 1**Fetal Brain Hybridization Intensities  
Poly(A) vs cDNA



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/22966

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/68 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| X          | WO 97 27317 A (CHEE MARK ;LAI CHAOQIANG (US); LEE DANNY (US); AFFYMETRIX INC (US))<br>31 July 1997<br>see the whole document<br>---  | 1-22                  |
| X          | ZHAO ET AL.: "HIGH-DENSITY cDNA FILTER ANALYSIS: NOVEL APPROACH FOR LARGE-SCALE, QUANTITATIVE ANALYSIS OF GENE EXPRESSION" GENE, vol. 156, 1995, pages 207-213, XP002094436<br>see the whole document<br>---<br>-/-- | 1-22                  |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 February 1999

Date of mailing of the international search report

12/03/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hagenmaier, S

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/22966

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|--|---|-----------------------|
| Category   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| X  | <p>TAKAHASHI ET AL.: "HIGH-DENSITY cDNA ANALYSIS OF THE EXPRESSION PROFILES OF THE GENES PREFERENTIALLY EXPRESSED IN HUMAN BRAIN" GENE, vol. 164, 1995, pages 219-227, XP002094437 see the whole document</p> <p style="text-align: center;">---</p>  | 1-22                  |
| X  | <p>SCHEMA M ET AL: "PARALLEL HUMAN GENOME ANALYSIS: MICROARRAY-BASED EXPRESSION MONITORING OF 1000 GENES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, no. 20, October 1996, pages 10614-10619, XP002022507 see the whole document</p> <p style="text-align: center;">---</p> | 1-22                  |
| X  | <p>BORMAN S: "DNA CHIPS COME OF AGE AFTER PERIOD OF GESTATION, TECHNOLOGY FOR GENETIC ANALYSIS IS BLOSSOMING" CHEMICAL AND ENGINEERING NEWS, vol. 74, no. 50, 9 December 1996, page 42/43 XP000633458 see the whole document</p> <p style="text-align: center;">---</p>                           | 1-22                  |
| X  | <p>LOCKHART ET AL.: "EXPRESSION MONITORING BY HYBRIDIZATION TO HIGH-DENSITY OLIGONUCLEOTIDE ARRAYS" NATURE BIOTECHNOLOGY, vol. 14, December 1996, pages 1675-1680, XP002094438 see the whole document</p> <p style="text-align: center;">---</p>  | 1-22                  |
| X  | <p>SCHEMA M ET AL: "QUANTITATIVE MONITORING OF GENE EXPRESSION PATTERNS WITH A COMPLEMENTARY DNA MICROARRAY" SCIENCE, vol. 270, no. 5235, 20 October 1995, pages 467-470, XP000644675 see the whole document</p> <p style="text-align: center;">---</p>   | 1-22                  |
| X  | <p>KAUPPINEN ET AL.: "CONTRIBUTION OF CYTOPLASMIC POLYPEPTIDES TO THE PROTON NMR SPECTRUM OF DEVELOPING RAT CEREBRAL CORTEX" MAGN. RESON. MED., vol. 25, no. 2, 1992, pages 398-407, XP002094505 See figure 3, page 404 see the whole document</p> <p style="text-align: center;">---</p>         | 3                     |
| A  | <p>WO 95 13374 A (BAYLOR COLLEGE MEDICINE) 18 May 1995 see the whole document</p> <p style="text-align: center;">---</p>  | 1-22                  |

-/--

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/22966

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|--|---|-----------------------|
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| A  | EP 0 717 105 A (PASTEUR INSTITUT)<br>19 June 1996<br>see the whole document<br>---  | 1-22                  |
| A  | WO 94 26905 A (US HEALTH ;NESTEC SA (CH))<br>24 November 1994<br>see the whole document<br>---  | 1-22                  |
| A  | US 5 569 588 A (ASHBY MATTHEW ET AL)<br>29 October 1996<br>see the whole document<br>---  | 1-22                  |
| A  | PATENT ABSTRACTS OF JAPAN<br>vol. 096, no. 005, 31 May 1996<br>& JP 08 009969 A (EISAI CO LTD),<br>16 January 1996<br>see abstract<br>---   | 1-22                  |
| P,X  | SEDLAK B J: "GENE CHIP TECHNOLOGY READY<br>TO IMPACT DIAGNOSTIC MARKETS"<br>GENETIC ENGINEERING NEWS, December 1997,<br>page 1, 14, 34 XP000749581<br>see the whole document<br>----- | 1-22                  |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/22966

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s)  | Publication<br>date  |
|---|---------------------|---|--|
| WO 9727317 A                              | 31-07-1997          | AU 2253397 A<br>EP 0880598 A  | 20-08-1997<br>02-12-1998   |
| WO 9513374 A                              | 18-05-1995          | US 5750367 A<br>AU 1052695 A<br>US 5798209 A  | 12-05-1998<br>29-05-1995<br>25-08-1998   |
| EP 0717105 A                              | 19-06-1996          | AU 4037395 A<br>CA 2165098 A<br>JP 8242866 A<br>NZ 280674 A                                 | 20-06-1996<br>15-06-1996<br>24-09-1996<br>22-09-1997                             |
| WO 9426905 A                              | 24-11-1994          | US 5506131 A<br>AU 697896 B<br>AU 6914594 A<br>CA 2163233 A<br>EP 0700442 A<br>US 5660986 A | 09-04-1996<br>22-10-1998<br>12-12-1994<br>24-11-1994<br>13-03-1996<br>26-08-1997 |
| US 5569588 A                              | 29-10-1996          | AU 6720996 A<br>CA 2202154 A<br>EP 0791078 A<br>JP 10507647 T<br>WO 9706277 A               | 05-03-1997<br>20-02-1997<br>27-08-1997<br>28-07-1998<br>20-02-1997               |